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ICTRED paper By Adriyan Pramono

 2nd International Conference on Tropical and Coastal Region Eco Development 2016 IOP Conf. Series: Earth and Environmental Science 55 (2017) 012058 IOP Publishing doi:10.1088/1755-1315/55/1/012058 International Conference on Recent Trends in Physics 2016 (ICRTP2016) Journal of Physics: Conference Series 755 (2016) 011001 IOP Publishing doi:10.1088/1742-6596/755/1/011001 Low zinc serum levels and high blood lead levels among school- age children in coastal area Adriyan Pramono1, Binar Panunggal1*, M.Zen Rahfiludin2, Fronthea Swastawati3 1Department of Nutrition/Center of Nutrition Research (CENURE), Faculty of Medicine, Diponegoro University, Jl.Dr.Sutomo No.18, Semarang 50231, Indonesia, adriyanpramono@fk.undip.ac.id 2 Faculty of Public Health, Diponegoro University, Jl. Prof.Sudarto SH, Semarang 50277, Indonesia, rahfiludin@yahoo.com 3 Faculty of Marine and Fisheries, Diponegoro University, Jl. Prof.Sudarto SH, Semarang 50277, Indonesia, fronthea_thp@yahoo.co.id *Email: panunggalbinar@gmail.com Abstract. The coverage of environmental lead toxicant was quiet wide. Lead exposure recently has been expected to be associated with zinc deficiency and blood indices disturbance. Emphasizing on children, which could absorb more than 50 % of lead that enters the body. Lead became the issue on the coastal area due to it has polluted the environment and waters as the source of fisheries products. This was a cross sectional study to determined nutritional status, blood lead levels, zinc serum levels, blood indices levels, fish intake among school children in coastal region of Semarang. This study was carried out on the school children aged between 8 and 12 years old in coastal region of Semarang. Nutritional status was figured out using anthropometry measurement. Blood lead and zinc serum levels were analyzed using the Atomic Absorbent Spectrophotometry (AAS) at a wavelength of 213.9 nm for zinc serum and 283.3 nm for blood lead. Blood indices was measured using auto blood hematology analyzer. Fish

intake was assessed using 3- nonconsecutive days 24-hours food recall. The children had high lead levels (median 34.86 µg/dl, range 11.46 - 58.86 µg/dl) compared to WHO cut off. Zinc serum levels was low (median 18.10 µg/dl, range 10.25 – 41.39 µg/dl) compared to the Joint WHO/UNICEF/IAEA/IZiNCG cut off. Approximately 26.4% of children were anemic. This study concluded that all school children had high blood lead levels, low zinc serum, and presented microcytic hypochromic anemia. This phenomenon should be considered as public health concern. Keywords: blood lead, zinc serum, coastal region, microcytic hypochromic anemia, school children 1. Introduction Zinc (Zn) is an essential trace element plays role as a cofactor of more than 100 metaloenzym, plays an important part in cell regeneration, metabolism, growth and immune function [1]. Zinc deficiency is associated with suboptimal growth, diarrhea, and decreased of immunity [2]. World Health Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI. Published under licence by IOP Publishing Ltd 1 2nd International Conference on Tropical and Coastal Region Eco Development 2016 IOP Conf. Series: Earth and Environmental Science 55 (2017) 012058 IOP Publishing doi:10.1088/1755-1315/55/1/012058 Organization (WHO) estimates the prevalence of zinc deficiency in the world's population is ranged between 4 and 73%. Additionally, about 5 to 30% zinc deficiency has occurred in children and adolescents both in developed and developing countries [3]. Especially in the developing countries, harmful environmental exposure recently has been associated with the deficiency of Zn. Nutritional zinc deficiency can be caused from lead (Pb) exposure through dangerous cycle that increases lead absorption and increases Zn secretion consecutively [4]. Previous studies in animal experiments summarized that the conditions of marginal Zn deficiency, could increase blood lead levels in the presence of Pb exposure [5,6]. Among school children, chronic Pb exposure is correlated with nutritional deficiencies, anemia, impairments to physical growth, cognitive deficient and learning disorders [7 - 10]. Anemia is a major public health problem especially in developing countries. Additionally, WHO reported that of 25,4% anemia has been found in school children [11]. Anemia is a state of decreasing of erythrocytes and hemoglobin, whereas those condition impaired its function to carry oxygen through body tissues sufficiently [12,13]. Hemoglobin is composed of four globin chain polypeptide whereas in each chain contains a heme molecule containing Iron [14]. As well as iron, zinc also plays an important role in the formation of hemoglobin. In heme biosynthesis, δ-ALA dehydratase enzyme that catalyzes charge 2 δ- ALA into molecules that is highly dependent on zinc [15]. A study conducted in Atlanta gives us information that there is a significant correlation between serum zinc concentrations and hemoglobin levels since children who suffered iron deficiency anemia tend to be deficiency of zinc serum [16]. Normal zinc level has a consequence on erythrocytes life period, due to zinc contributes in protecting erythrocytes from oxidative stress and cell damage [17]. Increasing of blood lead levels could also interfered with erythropoiesis by inhibiting the synthesis of protoporphyrin and the absorption of iron (Fe) thus increasing the risk anemia. Furthermore, lead also affects the morphology of erythrocytes [18]. Nowadays, rapid growth of industrialization and its waste became environmental issues in coastal region of Indonesia. The industrial waste that was dumped in the coastal region induced the increasing level of lead pollutant in this area [19]. The coverage of environmental lead toxicant was quiet wide, including the exposure of fisheries products, public water source, and air pollution [20]. Lead exposure among children is an increasing problem globally, operated using the Atomic Absorbent Spectrophotometry (AAS) at a wavelength of 213.9 nm for zinc serum and 283.3 nm for blood lead (Shimadzu AA6401F, Japan). Biochemical measurement was organized in the micronutrient and IDD Center laboratory, Diponegoro University, Indonesia. Dietary assessment was conducted to determine fish intake among this population. The three- non consecutive days 24-hour food recall method was carried out to describe fish intake. In this study, local fish products have also been collected from this coastal area. Environmental assessment has been conducted on drinking water samples were collected using standard methods. Lead contents from fish products was measured using Spectrophotometry. Lead analysis of local fish products and drinking water samples were carried out in Center of Prevention on Industrial Pollution Laboratory, Ministry of Industrial, Republic Indonesia. 2.3. Statistical analysis The SPSS for IBM version 19 and Microsoft excel software were performed for statistical analysis. All the study variables were tested for normality by the Kolmogorov-Smirnov test. The Mann Whitney test was operated to compare the blood lead levels and zinc serum levels between subjects with anemia and non anemia. The Correlation between blood lead level and serum zinc, hb, ht, RBC, mcv, mch, and ferritin were performed using Spearman rank's correlation analysis. Statistical significance was considered when p value < 0.05. 2.4. Ethics The study protocol was approved by the Board of Medical Ethics on Faculty of Medicine Diponegoro University / Dr. Kariadi Hospital with no. 534/EC/FK-RSDK/2015. The participants, who were recruited in this study, clearly informed about the purpose of investigations and expected outcomes. Informed consent was obtained and signed by the parents of the subjects before the study began. 3 2nd International Conference on Tropical and Coastal Region Eco Development 2016 IOP Conf. Series: Earth and Environmental Science 55 (2017) 012058 IOP Publishing doi:10.1088/1755-1315/55/1/012058 3. Results 3.1. Demographic characteristics of respondents The coastal region in this study was taken place at Tambak Lorok area, the northern area of Semarang, Central Java. This area borders along with java sea. The main harbor of Central Java province has been located in this coastal area. Industrial area that was built in this region may contributed to environmental and health problems issue. Figure 1 depicts environmental situation in this coastal region. Figure 1. Environmental situation of Semarang coastal region (authors documented). 3.2. Biochemical data analysis Table 1 shows values of haemoglobin, hematocrit, red blood indices, zinc serum levels, blood lead levels among all children. All subjects had blood lead levels > 10 (μg/dl), low zinc serum levels (< 65 μg/dl), and median of ferritin describes < 15 ng/ml. Table 1. Mean, SD, median, minimum and maximum values of blood indices, zinc serum, blood lead levels, and ferritin among subjects (N=72) Biochemical parameters Median Range (min – max) Haemoglobin (g/dl) Hematocrit (g/dl) RBC (×106 mm3) MCV(μ3) MCH MCHC Ferritin (ng/ml) Zinc serum (μg/dl) Blood Lead Level (μg/dl) 12.85 35.40 4.82 74.45 27.00 36.30 13.56 18.10 34.86 10,00 – 15,6 28,70 – 44,70 4.21 – 6.00 52.10 – 81.05 18.10 – 29.70 34.10 – 38.30 2.28 – 44.68 10,25 – 41,39 11.46 – 58.86 This study was carried out to 72 children with age range from 9 to13 years with a mean value of 10.16 ± 1.02 years. Approximately 66.7% of children's age were categorized between 9 and 10 years old. 4 2nd International Conference on Tropical and Coastal Region Eco Development 2016 IOP Conf. Series: Earth and Environmental Science 55 (2017) 012058 IOP Publishing doi:10.1088/1755-1315/55/1/012058 Surprisingly, about 90.3% of children were stunting, consists of mild stunting 34.7%, moderate stunting 45.8%, and severe stunting 9.7%. We can see from table 1 about 26.4% children were anemic. Father's occupation of subjects largely as fisherman (59.7%). Both father and mother of children had literate, mostly graduated from Senior High School (father 51.4%, mother 45.8%) (Table 2). Table 2. Characteristics of school age children (N=72) Characteristics N (%) Sex Male Female Age 9 – 10 11 – 12 Height for Age Nutritional Status Normal Mild Stunting Moderate Stunting Severe Stunting Anemia Status Anemic Non Anemic Father literacy Graduated elementary school Graduated junior high school Graduated senior high school Mother literacy Graduated elementary school Graduated junior high school Graduated senior high school Father's occupation Salesman Fisherman Labor Mother's occupation Household Salesman Labor 39 (54.2) 33 (45.8) 48 (66.7) 24 (33.3) 7 (9.7) 25 (34.7) 33 (45.8) 7 (9.7) 19 (26.4) 53 (73.6) 13 (18.1) 22 (30.6) 37 (51.4) 16 (22.2) 23 (31.9) 33 (45.8) 5 (6.9) 43 (59.7) 24 (33.3) 36 (50.0) 2 (2.8) 34 (47.2) Comparison between mean values of different hematological parameters, blood lead levels, zinc serum levels and serum ferritin in anemic and non anemic were figured out in this study (Table 3). Regarding the hematological parameters, nearly all values were significantly lower among the anemic than the non- anemic group except for blood lead levels, which showed a highly significant elevation among the anemic group. However, according to World Health Organization (WHO) cut off for blood lead levels, all children in this study had a blood lead ≥10 µg/dl (high blood lead level group {HBLLs}). As for the RBC count, ferritin, and zinc serum, no statistically significant difference was detected between the groups. 5 2nd International Conference on Tropical and Coastal Region Eco Development 2016 IOP Conf. Series: Earth and Environmental Science 55 (2017) 012058 IOP Publishing doi:10.1088/1755-1315/55/1/012058 Table 3. Comparison between mean values of different hematological parameters, zinc serum, blood lead levels, and serum level of ferritin in anemic and non-anemic groups. Biochemical Anemic Group (N=19) Non Anemic Group (N=53) Test of parameters Significance RBC (×106 mm3) 4.82 + 0.49 4.88 + 0.33 t = - 0.56 p = 0.58 Hb (g/dl) 11.65 + 0.62 13.26 + 0.77* t = - 9.03 p = 0.000 Hct 32.66 + 1.44 36.47 + 1.88* t = - 9.05 p = 0.000 MCV(µ3) 68.43 + 7.50 74.89 + 3.74* t = - 4.84 p = 0.000 MCH 24.45 + 3.05 27.26 + 1.65** t = - 5.01 p = 0.000 MCHC 35.66 + 0.92 36.39 + 0.75* t = - 3.09 p = 0.005 Ferritin (ng/ml) 15.59 + 8.39 15.36 + 8.58 t = 0.10 p = 0.92 Zinc serum (µg/dl) 20.13 + 9.64 20.44 + 8.31 t = - 0.12 p = 0.90 Blood lead level (µg/dl) 41.95 + 10.61 32.03 + 10.33** t = 3.52 p = 0.001 *independent t-test were performed (significant if p<0.05) **Mann Whitney test were performed (significant if p<0.05) Table 4 reveals the correlation between the different red blood indices, serum zinc levels and the blood levels of lead. Blood lead levels had a significant negative correlation with haemoglobin, hematocrit, and zinc serum (r = -0.330, r = -0.328, respectively p<0.005 and r = -0.265, p < 0.05). Table 4. Correlation of different hematological parameters, serum iron and ferritin levels in relation to blood lead, copper and zinc. Hematological parameters Blood lead levels Zinc serum levels r-value (p-value) r-value (p-value) RBC (×106 mm3) -0.066 (0.585) -0.128 (0.285) Hb (g/dl) -0.330 (0.005) 0.119 (0.319) Hct -0.328 (0.005) 0.101 (0.396) MCV(µ3) -0.124 (0.298) 0.130 (0.276) MCH -0.109 (0.363) 0.148 (0.214) MCHC -0.106 (0.375) 0.144 (0.227) Ferritin (ng/ml) 0.178 (0.135) 0.075 (0.529) Zinc serum levels -0.265 (0.025) Blood lead levels - - -0.265 (0.025) 3.3. Environmental assessment Environmental assessment has been conducted on drinking water samples and dietary fish intake. Local fish products were collected based on the information from dietary fish intake interview. Lead content of drinking water and local fish products can be seen in Table 5. 6 2nd International Conference on Tropical and Coastal Region Eco Development 2016 IOP Conf. Series: Earth and Environmental Science 55 (2017) 012058 IOP Publishing doi:10.1088/1755-1315/55/1/012058 Table 5. Fish intake (g per day) and lead levels of drink water and local fish products Parameters Mean Median Range (min – max) Fish intake (g/day) 40 10,0 – 128 Lead from drinking water 0 0 (μg/dl) Lead from some type of fish (mg/Kg) - Green mussels (Pena 1.13 viridis) - Scallop shell (Anadara 0.76 granosa) - Kipper fish 0.18 (Scatophagidae) - Snapper fish (Lates 0.13 calcalifer) - Mullets fish (Mugil 0.18 cephalus) 4. Discussion All school children in this study (100%) had BLL ≥ 10 μg/dl and zinc serum levels < 65 μg/dl, similar to a study done by Hegazy et al [7]. who also reported about more than a half subjects (63.3%) had BLL > 10 μg/dl. However the current study is similar to the results obtained for children population in India [24]. The cut off value of l0 μg/dl defined by the WHO guideline for Childhood Lead Poisoning as a limit for an elevated blood lead level primarily is based on neurological toxicity and has wide range of toxicity including neurobehavioral impairment [21]. Recently, even though blood lead levels (BLL) less than 10 μg/dl is considered safe, a study confirmed that BLL < 10 μg/dl has associated with cognitive deficits [25]. Thus our data showed all children had low zinc serum levels (< 65 μg/dl). The Joint WHO/UNICEF/IAEA/IZiNCG asserted zinc serum levels < 65 μg/dl has been recognized as serious public health problems [26]. Schwartz et al [28] reported that children living near lead smelters in the US of Idaho, had blood lead levels approximately 25 μg/dl and were correlated with anaemia. In addition, Jain et al [29]. reported that children with BLL >10 μg/dl had 1.7 times risk to be moderate anaemia, in contrast with our finding showed that 73.6% of children had normal haemoglobin. Froom et al [30]. suggested that haemoglobin level did not correlate well with BLLs. However, our finding described that Hb, Hct, MCV, MCH and MCHC values of children with anaemia lower in comparison to anemic group. Moreover high BLLs has negatively associated with lower zinc serum and some blood indices levels. Lead absorption occurs predominantly in the duodenum and jejunum. The process of lead entry into the body may carry on through food, drink or by air. Approximately 90 % of lead absorbed by the blood binds to red blood cells [27]. The children absorbed up to 50% of lead from food and/or drink through the gastrointestinal tract and will be included in the metabolic processes of the body. Those lower red blood indices is similar with hypochromic microcytic anaemia which can be caused by blood lead toxicity [7]. Lead causes anaemia by impairing heme synthesis and increasing the rate of red blood cell destruction [31]. Although a causal pathway cannot be determined, yet the study findings clearly demonstrate the differences of BLLs between anaemia status. In the present study zinc serum level of the anemic group is insignificantly different than non- anemic group. Lead (Pb) has the same valiancy number (Pb2+) as zinc (Zn2+) and iron (Fe2+), whereas on the cellular transport by Dimetal Transporter-1 (DMT-1) competition may be occurred [32]. Lead (Pb2+) will always be dominant to carry on by DMT-1 because of its density higher than Zn2+ and Fe2+ 7 2nd International Conference on Tropical and Coastal Region Eco Development 2016 IOP Conf. Series: Earth and Environmental Science 55 (2017) 012058 IOP Publishing doi:10.1088/1755-1315/55/1/012058 [27]. Lead (Pb) inhibits δ-ALA dehydratase enzyme that catalyzes δ-ALA into molecules, which is highly dependent to zinc on haemoglobin synthesis [15]. In the current study high BLLs among school children may be due to air pollution, sewage contamination, and probably from food consumption which were contaminated with Lead (Pb). Based on dietary interview, children consume fish regularly (range between 10 g/day and 128 g/day). Thus based on lead measurement content on some fish products showed variety lead content (mg/Kg fish products), green mussels (Pena viridis) had the highest content of lead (1.13mg/Kg). Green mussels (Pena viridis) has been vended on street food vendor near by school building. However a causal pathway between some fish products intake and blood lead levels cannot be firmed only from this data. A large epidemiology study on general northern coastal communities should remain a concern because of the nature of accumulation. In developing countries such as Indonesia, control of lead contamination is much slower and the negative health effects getting more sporadic. The present work revealed that lead contamination should be considered as public health concern in northern coastal population of Semarang, Central Java, Indonesia. 5. Conclusion In the present study, all subjects has high blood lead levels (BLLs) and low zinc serum. Blood lead levels (BLLs) were negatively associated with the hematological parameters and zinc serum levels. Lead (Pb) was not presented in drinking water, but Pb was discovered in some fish products that may be regularly consume by the children. Lead exposure could be controlled and strides should be taken to reduce zinc deficiency and anaemia among children population at coastal region. 6. References [1]. Osredkar J, Sustar N. 2011. Copper and zinc, biological role and significance of copper/zinc imbalance. J Clin Toxicol Suppl 3, 1 – 18. [2]. Prasad AS. 2003. 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